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NeuroImage 23 (2004) 296–304

Concurrent pharmacological MRI and in situ microdialysis of cocaine reveal a complex relationship between the central hemodynamic response and local dopamine concentration ☆

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Received 19 February 2004; revised 8 April 2004; accepted 3 May 2004
Available online 20 July 2004

The mechanisms underlying the signal changes observed with pharmacological magnetic resonance imaging (phMRI) remain to be fully elucidated. In this study, we obtained microdialysis samples in situ at 5-min intervals during phMRI experiments using a blood pool contrast agent to correlate relative cerebral blood volume (rCBV) changes with changes in dopamine and cocaine concentrations following acute cocaine challenge (0.5 mg/kg iv) in the rat over a duration of 30 min. Three brain areas were investigated: the dorsal striatum (n = 8), the medial prefrontal cortex (mPFC; n = 5), and the primary motor cortex (n = 8). In the striatum and mPFC groups, cocaine and dopamine temporal profiles were tightly correlated, peaking during the first 5-min period postinjection, then rapidly decreasing. However, the local rCBV changes were uncorrelated and exhibited broader temporal profiles than those of cocaine and dopamine, attaining maximal response 5–10 min later. This demonstrates that direct vasoactivity of dopamine is not the dominant component of the hemodynamic response in these regions. In the motor cortex group, microdialysis revealed no local change in dopamine in any of the animals, despite large local cocaine increase and strong rCBV response, indicating that the central hemodynamic response following acute iv cocaine challenge is not driven directly by local dopamine changes in the motor cortex. The combination of phMRI and in situ microdialysis promises to be of great value in elucidating the relationship between the phMRI response to psychoactive drugs and underlying neurochemical changes.

Keywords: Pharmacological magnetic resonance imaging; Microdialysis; Dopamine

Introduction

Magnetic resonance imaging (MRI) methods can be applied to study the effects of pharmacological agents on the spatiotemporal patterns of brain activity in humans (Breiter et al., 1997; Stein et al., 1998) and laboratory animals (Chen et al., 1997; Jenkins et al., 2003; Marota et al., 2000; Xu et al., 2000). More specifically, the pharmacological MRI (phMRI) approach tracks signal changes reflecting the central hemodynamic response induced by acute pharmacological challenge (Jenkins et al., 2003). Different pharmacological tools have been used to activate neurotransmitter systems and to study the modulatory action of more selective receptor agonists and antagonists (Jenkins et al., 2003; Leslie and James, 2000; Morris, 1999). However, the mechanisms underlying the signal changes observed by phMRI and their correlation with modulations in neurotransmitter concentrations remain to be fully elucidated.

Cocaine produces a widespread central hemodynamic response (Stein and Fuller, 1992, 1993) and has been widely used in phMRI studies in the rat (Luo et al., 2003; Mandeville et al., 2001; Marota et al., 2000), where intravenous (iv) administration results in temporal profiles that vary with anatomical location (Marota et al., 2000). One of the primary actions of cocaine is dopamine transporter blockade, leading to increased intrasynaptic dopamine. These modulations in the mesolimbic dopamine system are thought to be a fundamental mechanism underlying the psychostimulant effects and reinforcing properties of cocaine and other drugs of abuse (Koob and Le Moal, 1997). Other pharmacological stimuli affecting the dopaminergic system [amphetamine and CFT (2β-carbomethoxy-3β-(4-fluorophenyl)tropane)] have been shown to yield rCBV changes that correlate in amplitude (Chen et al., 1997) and temporal profile (Chen et al., 1999) in the rat striatum with changes in dopamine following intravenous challenge. This led to the hypothesis that dopamine release and the hemodynamic response are tightly cou-

☆ Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neuroimage.2004.05.001.

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Available online on ScienceDirect (www.sciencedirect.com.)

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doi:10.1016/j.neuroimage.2004.05.001

pled, with relative cerebral blood volume (rCBV) changes driven by the dopamine concentration (Chen et al., 1997, 1999; Jenkins et al., 2003). This could reflect increased metabolic demand following neural activation at dopaminergic synapses. However, dopaminergic neurons also project postsynaptically onto the vasculature (Goldman-Rakic et al., 1992; Williams and Goldman-Rakic, 1998), and dopamine itself has a rapid and direct vasoactive action (Edvinsson and Krause, 2002; Krimer et al., 1998). The substantial attenuation of the (rCBV) phMRI response to cocaine (Marota et al., 2000) and amphetamine (Choi et al., 2003) by selective dopamine D_1 receptor blockade, and the observation that dopamine $D_{1/5}$ receptors are expressed on microvessels and capillaries in the cortex (Choi et al., 2003), suggested that direct action on dopamine D_1 receptors on the cerebral vasculature may be a major component of the hemodynamic response to dopaminergic ligands (Choi et al., 2003; Jenkins et al., 2003). Under the hypothesis that dopamine release and the central hemodynamic response to cocaine are tightly coupled, interregional differences in phMRI time courses (Marota et al., 2000) might reflect different temporal profiles of dopamine concentration. Moreover, interanimal variability in the amplitude of the phMRI response could indicate an underlying variability in neurotransmitter changes.

To test these hypotheses and determine more fully the relationship between the central hemodynamic response and modulations in dopamine concentration following cocaine challenge, we established methodology enabling in situ microdialysis sampling during the phMRI experiment. Cocaine has relatively fast pharmacokinetics, with an intravenous plasma half-life of 15–20 min in the rat (Stein and Fuller, 1993), and differences in rCBV profile occur primarily during the first 10–15 min (Marota et al., 2000). The time resolution of the microdialysis sampling was thus increased to 5 min to approximate more closely the time scale of rCBV changes. A specific and highly sensitive liquid chromatography or mass spectrometry assay (LC/MS-MS) was developed to provide the concurrent determination of the cocaine and dopamine levels in the collected microdialysis samples. This enabled analysis of simultaneous dynamic changes in the concentrations of cocaine ([CA]) and dopamine ([DA]) and the rCBV component of the hemodynamic response within the same animals. We focused this study on three structures: the dorsal striatum (dStr), primary motor cortex (M1), and medial prefrontal cortex (mPFC). Each of these regions shows a different rCBV temporal response profile, and each has a different relationship with the major dopaminergic system projections (Fallon and Moore, 1978; Groenewegen et al., 1997; Heidbreder and Groenewegen, 2003; Steketee, 2003; Wu et al., 2003). Microdialysis samples were obtained from either the striatum, motor cortex, or mPFC concurrent with rCBV-phMRI acquisition, enabling correlation of cocaine and dopamine modulation with rCBV changes within the same animal.

Materials and methods

All experiments were carried out in strict accordance with Italian animal welfare legislation and GSK internal ethical review.

Microdialysis surgery

Animals were divided into three groups, with the microdialysis probe tip located in either the dorsal striatum (dStr; $n = 8$), medial prefrontal cortex (mPFC; $n = 5$), or motor cortex (M1; $n = 8$). Rats were anesthetized with a mixture of medetomidin and zolazepam and placed in a stereotaxic apparatus for small animals (David Kopf). A vertical, nonmetallic guide cannula (Agntho's, Sweden) was inserted through a 1-mm hole drilled on the skull. The coordinates with respect to bregma were as follows (Paxinos and Watson, 1998): dorsal striatum: anterior (A) +1.0 mm; ventral (V): −3.1 mm; lateral (L) +2.5 mm; mPFC (IL/PrL) A +2.7 mm; V −2.5 mm; L +0.5 mm; Motor Cortex A +2.2 mm; and V −1 mm; L +2.8 mm. The guide cannula was secured with epoxy glue and two plastic screws were anchored onto the skull at two additional holes. Rats were then singly housed for 6–8 days with food and water available ad libitum.

Microdialysis acquisition

Following 6–8 days recovery, the animals were prepared for the MRI experiment. The cannula stylette was removed and a nonmetallic probe with a 2-mm-long cuprophane membrane (Agntho's) was inserted into the guide cannula. The probe inlet was connected to a 3-m PE 10 tube connected to a gas-tight syringe on a microinfusion pump. Artificial cerebrospinal fluid (KCl 2.5 mM, NaCl 125 mM, $CaCl_2$ 1.3 mM, $MgCl_2$ 1.18 mM, $Na_{2H}PO_4$ 2 mM, pH 7.4, with H_3PO_4 85%) was pumped through the probe at a steady flow rate of 2.0 μl/min. The outlet tubing was 60 cm long and approximately 1 1/2 h of perfusion was allowed before drug challenge. Samples were collected in microcentrifuge tubes every 5 min and immediately frozen at −80 °C for further analysis of dopamine and cocaine levels through mass spectrometry technique. Ten baseline samples were acquired before cocaine challenge, which was timed to coincide with sample changeover. At least five postchallenge microdialysis samples were acquired.

Basal [DA] was calculated for each animal as the average of the nine samples collected from −45 to −5 min relative to cocaine injection. Subsequent [DA] values were expressed as percentage of these baseline values. Cocaine measures were retained as absolute concentrations, as baseline values were zero.

Dopamine and cocaine quantification assay

A liquid chromatography or mass spectrometry assay was developed to measure simultaneously extracellular levels of cocaine and dopamine. All LC/MS-MS measurements were performed using an Agilent 1100 HPLC system coupled with a Quattro Ultima triple-quadruple mass spectrometer (Micromass, Manchester, UK). Analytes were separated on a Supelco Discovery HS C18 (2.1 mm id, 15 cm total length) column, using a 4-min fast gradient: 2 min of isocratic 100% of A, then from 0% to 95% of B in 2 min, and finally isocratic 95% of B for 3 min (A: H_2O + 0.5% acetic acid, and B: MeOH + 0.5% acetic acid + 0.01% triethylamine). The column temperature was maintained at 35 °C. All analytes were ionized in the ESI interface in the positive ion mode and detected using multiple reaction monitoring (MRM). The MRM transitions m/z 154.0 > 137.1 and 304.0 > 181.9 were sequentially monitored for the detection of dopamine and cocaine, respectively. The electrospray capillary voltage was set at 3.0 kV, cone voltage at 35 V, source temperature at 120 °C, and desolvation temperature at 220 °C. The collision gas flow (argon) was adjusted to achieve a pressure of 1.3×10^{-3} mbar in the collision cell. All the ion source and MS parameters were optimized for each analyte by infusing of standard solutions using a Harvard syringe pump (Harvard Apparatus, Holliston, MA, USA). All data were processed by Micromass MassLynx 4.0 software.

Animal preparation for MRI

For preparation of the combined MRI or microdialysis experiment, the animals were anesthetized with 3% halothane in a 30%:70% O_2:N_2 gas mixture, tracheotomized and artificially ventilated with a mechanical respirator. Upon tracheotomy and throughout surgery, the anesthetic level was maintained at 1.5%. The left femoral artery and vein were cannulated (PE50 catheters) and the animal was then positioned prone in a customized stereotactic holder designed to accommodate the rat and the MRI receive coil. Animal paralysis was achieved by a single bolus of D-tubocurarine (0.25 mg/kg) through the vein and a continual infusion of D-tubocurarine (0.25 mg/kg/h) through the artery. Ventilation parameters were adjusted to keep the arterial blood gases values within physiological range ($32.6 < pCO_2 < 44$; $78.7 < pO_2 < 223$). During the image acquisition, the halothane anesthetic was set to 0.8% maintenance level. We used halothane during the functional acquisition as this anesthetic has been shown to preserve dopamine activity (Jenkins et al., 2003) and results in a robust phMRI response to cocaine (Mandeville et al., 2001; Marota et al., 2000). Mean arterial blood pressure (MABP) and heart rate were monitored continually throughout the experiment by means of a blood pressure transducer (Biopac Systems Corp., Goweta, USA) inserted in the femoral artery via the catheter.

Magnetic resonance imaging

MRI data were acquired using a Bruker Biospec 4.7T system, a 72-mm birdcage resonator for radiofrequency transmit and a Bruker "rat brain" quadrature receive coil secured to the animal holder above the head (Bruker Biospin, Ettlingen, Germany). This receive coil includes a central opening, enabling the microdialysis probe to remain in situ and samples to be acquired concurrently with the MRI acquisition. Following T_2-weighted anatomical imaging (matrix 256 × 256, field of view 40 mm, slice thickness 1 mm), the phMRI time series acquisition (Reese et al., 2000) composed a sequence of T_2-weighted RARE image volumes, with RARE factor 32, matrix 128 × 128, field of view 40 mm, slice thickness 2 mm, eight contiguous slices, $TE_{eff} = 110$ ms, TR = 2700 ms. Sequential image volumes ($N_t = 384$) were acquired at a time resolution of $\delta t = 10$ s, for a total phMRI acquisition time of approximately 64 min. To sensitize the signal intensity to changes in rCBV component of the hemodynamic response, a 2.67 mg/kg dose of the blood pool contrast agent "Endorem" (Guerbet, France) was administered intravenously following five reference image frames.

A 0.5 mg/kg iv bolus of cocaine (Sigma) in a total volume of 1.4 ml was administered approximately 30 min later over a duration of 60 s. This dose has been established as providing a widespread central rCBV response, uncorrelated to transient blood pressure changes (Mandeville et al., 2001; Marota et al., 2000).

MRI data analysis

The T_2-weighted anatomical images from each subject were coregistered by rigid body alignment with the transformation parameters for each animal subsequently applied to the time series data using the AFNI software package (Cox and Hyde, 1997). For comparison with the microdialysis results, time courses were extracted from regions of interest (ROIs) using the anatomical images, based on correspondence with an anatomical reference atlas (Paxinos and Watson, 1998). ROIs were located in the dStr, M1, and the infralimbic (IL) and prelimbic (PrL) subregions of the mPFC. In the former two locations, ROIs ipsilateral and contralateral to the probe were extracted, while the mPFC ROI encompassed both hemispheres (Fig. 1A).

The changes in MRI signal intensity in each ROI time course were then converted into rCBV changes using the log transform (Mandeville et al., 1998)

$$\mathrm{rCBV}(t) = \frac{\ln[S(t)/B(t)]}{\ln[B(t)/S_{\mathrm{PRE}}]}, \tag{1}$$

where $S(t)$ is the measured signal, S_{PRE} is the signal intensity before administration of the contrast agent, and $B(t)$ is the estimated background signal in the absence of transient functional stimuli. The contrast agent used here gives rise to a gradual animal-dependent signal increase as it is eliminated from the bloodstream. In the animals employed in this study, the mean half-life was 241 min, with most values between 120 and 260 min, but with 20% showing slower elimination with half-lives between 300 and 600 min. If not corrected for in the transformation of Eq. (1), this manifests as a negative drift in the rCBV time course. To enable more reliable within-animal comparison with dopamine changes, $B(t)$ was thus estimated for each time course from a constrained exponential fit to the baseline time points from 5 min following contrast administration to 2 min before cocaine challenge (approximately 140 time points, or 22 min). This method models the physiological elimination of the contrast agent from the bloodstream and has been demonstrated to provide robust and accurate estimation of the background signal over the 30-min duration of the postchallenge analysis (Schwarz et al., 2003). For direct comparison with the microdialysis data, each rCBV time course was also rebinned to 5-min temporal resolution.

Statistical data analysis

The similarity of the temporal profiles (cocaine vs. dopamine, rCBV vs. dopamine, rCBV vs. cocaine, and ipsilateral vs. contralateral rCBV) was evaluated for each cohort by the cross-correlation coefficient (at lag 0)

$$\rho = \frac{\sum_{k=0}^{N-1}(x_k - \bar{x})(y_k - \bar{y})}{\sqrt{\left[\sum_{k=0}^{N-1}(x_k - \bar{x})^2\right]\left[\sum_{k=0}^{N-1}(y_k - \bar{y})^2\right]}} \tag{2}$$

of the respective time series at 5 min resolution, using the eight time points from 0 to 30 min. This was performed both on group mean time courses and within individual animals. Stability of baseline dopamine concentrations was assessed by two-way ANOVA over the baseline values with time and subject as factors. Postchallenge values of [CA], [DA], and rCBV at individual 5-min time points were compared with baseline values using Student's t test, corrected for multiple comparisons using the Benjamini–Hochberg method with a false discovery rate of $q = 0.05$. Correlations in the amplitude of response (cocaine vs. dopamine, rCBV vs. dopamine, and rCBV vs. cocaine) were assessed from area under the curve (AUC) measures for [0,15]-min and [15,30]-min time windows. These windows were chosen because the different dynamic changes of the responses occurred primarily during the first 15 min, followed by a slower washout phase. The first window thus allowed us to assess

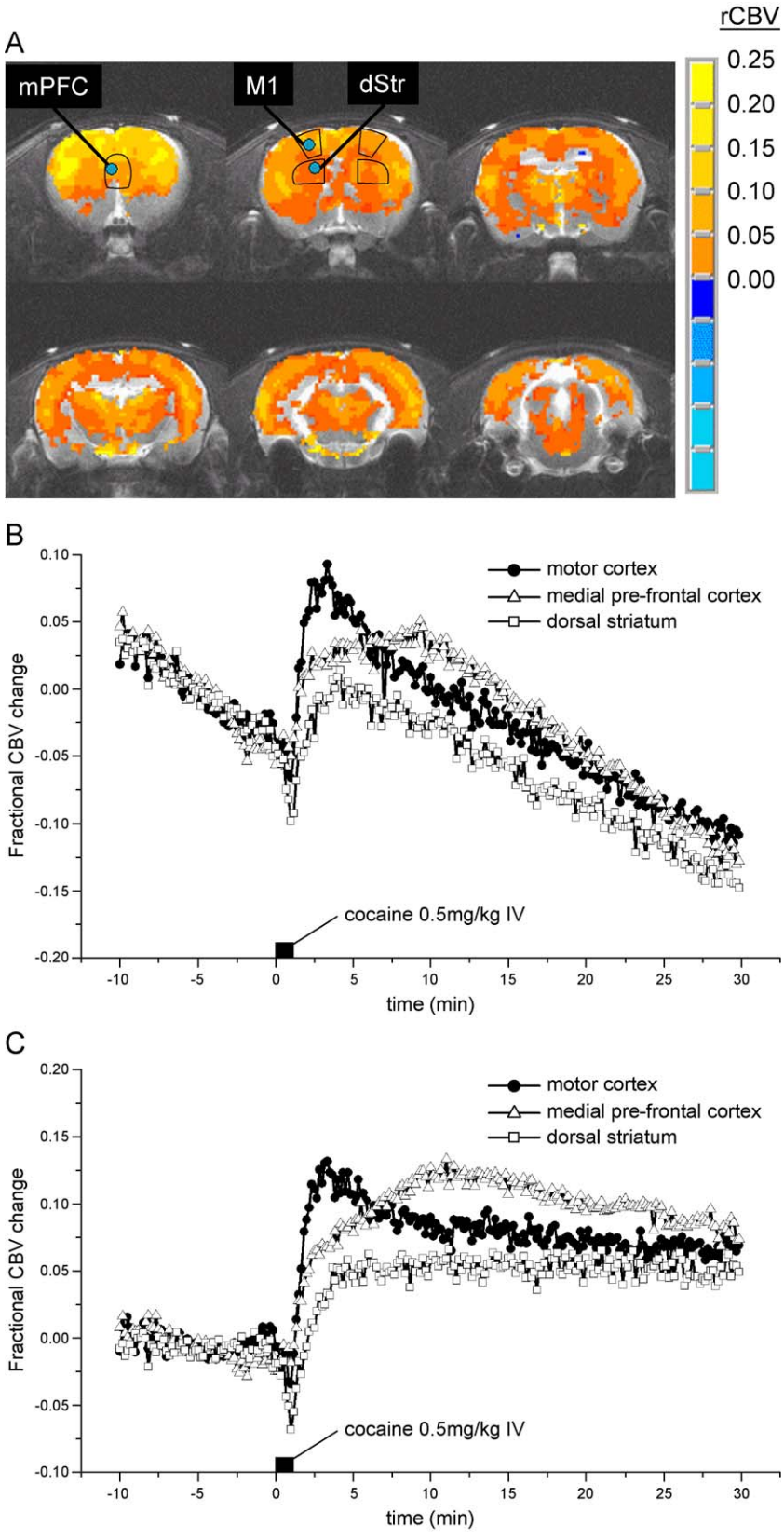

Fig. 1. (A) Map of rCBV response (AUC) following 0.5 mg/kg iv cocaine challenge, with the approximate locations of the microdialysis probe and ROIs for the three cohorts indicated. Numbers at the bottom-left of each slice indicate the approximate central position (in mm) of each slice relative to z-bregma. (B and C) Regional differences in temporal profile of rCBV response in the three anatomical regions examined (all cohorts combined, $n = 21$). The curves in (B) represent rCBV profiles calculated without detrending (i.e., assuming a constant baseline signal). Those in (C) were calculated using baseline estimation as described in the text.

any amplitude correlation between the initial responses, whereas the second explored the relationship between a subsequent sustained response or return to baseline.

Results

phMRI temporal profiles

Fig. 1A illustrates the spatial pattern of the rCBV response to cocaine challenge, showing rCBV increases in many brain regions, including those outside the mesolimbic system. Indeed, particularly strong CBV increases were observed in the frontal or parietal cortices and thalamus, with structures of high dopaminergic innervation such as the striatum and accumbens typically responding more weakly. Mean rCBV time courses from the dorsal striatum, mPFC, and motor cortex are shown in Figs. 1B and C. The regional dependence of these time courses is consistent with previously published phMRI observations (Marota et al., 2000). The response in the mPFC followed a steep initial gradient that slowed to a broad peak after approximately 10 min. The striatal rCBV increase was weaker, rising to peak value after approximately 5 min before following a broad plateau. The motor cortex showed a rapid rCBV increase to a peak at approximately 3 min before decreasing to a slower signal washout. The rapid and transient decrease in rCBV immediately upon cocaine injection, apparent in the striatum group time course, was also observed in other brain regions in individual animals, including the motor cortex. The time courses corrected for contrast agent washout in Fig. 1C closely resemble those reported for rCBF changes (Stein and Fuller, 1993), which also show a sustained response to 45 min postchallenge.

As in previously reported studies (Marota et al., 2000), blood pressure variations did not correlate with the phMRI time courses; typically, a transient MABP increase immediately following cocaine administration was observed, lasting about 1 min, before returning to baseline.

The location of the implanted cannula was visible on the high-resolution anatomical images (Fig. 2) but did not appear to greatly affect the local phMRI signals. The temporal profiles of ipsilateral and contralateral ROI time courses were strongly correlated in both the striatum and the motor cortex (Table 1) (the mPFC ROI was bilateral).

In the striatum and mPFC, dopamine and rCBV follow different temporal profiles

In the striatum group, both [CA] and [DA] peaked during the first 5-min period following injection before decreasing rapidly to baseline (Fig. 3). The temporal profiles of [CA] and [DA] were tightly correlated (Table 1). However, the [DA] profile did not reflect the time course of rCBV change, which evidenced a slower increase to a broad plateau and remained above baseline values for more sustained periods. This lack of positive covariance between dopamine and rCBV profiles is reflected in negative cross-correlation coefficients (Table 1).

In the mPFC group, [CA] followed a slightly broader profile than in the other two groups, peaking at 5–10 min, while [DA] again peaked during the first 5-min period postinjection (Fig. 4). The [CA] and [DA] profiles were again highly correlated (Table 1). As in the striatum group, the dopamine changes did not reflect the temporal profile of the rCBV response, which again evidenced a slower evolution, peaking between 10 and 15 min after injection and returning more slowly to baseline. Cross-correlation coefficients were correspondingly low (Table 1).

Amplitude correlations revealed a significant negative correlation between rCBV and [DA] changes in the [0,15]-min window in the mPFC group ($r = -0.81$, $P < 0.05$), with a nonsignificant negative trend ($r = -0.64$, $P = 0.24$, NS) in the later window. In the striatum group, a nonsignificant positive trend was observed in the [0,15]-min window ($r = 0.57$, $P = 0.14$, NS). No other significant correlations were found. In the striatum group, trends toward negative correlation between [CA] and [DA] in the

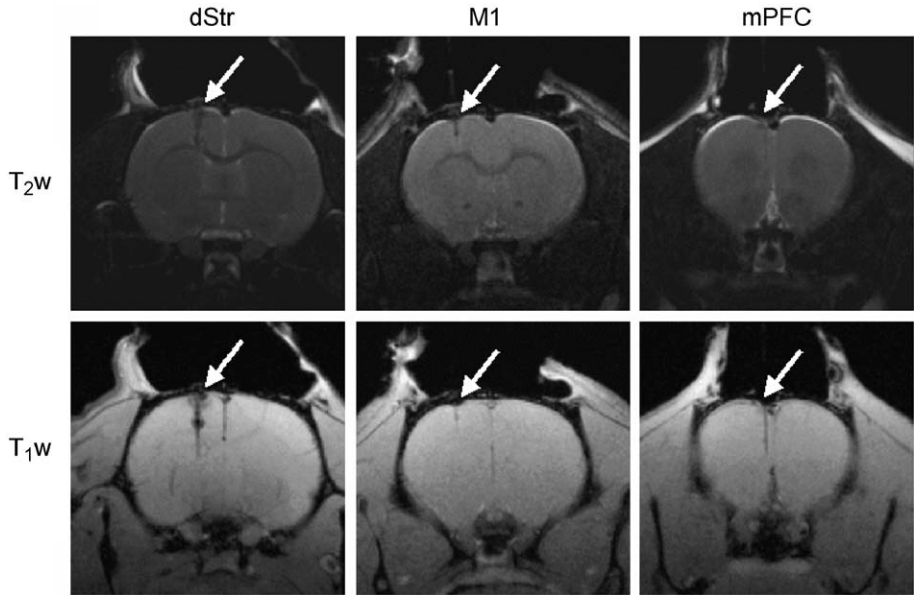

Fig. 2. High-resolution anatomical T_2w (RARE) and T_1w (gradient echo) images through the position of the microdialysis probe in one animal from each of the three cohorts (N.B. the slice thickness in these images is 1 mm, compared to 2 mm in the time series acquisition).

Table 1
Cross-correlation between time courses

Region	Comparison	ρ (group mean data)	ρ (within-animals; mean ± SEM)
dStr	[DA] vs. [CA]	0.97	0.78 ± 0.12
	rCBV vs. [DA]	−0.57	−0.32 ± 0.16
	rCBV vs. [CA]	−0.57	−0.20 ± 0.18
	ipsi- vs. contra-rCBV	0.98	0.69 ± 0.13
mPFC	[DA] vs. [CA]	0.89	0.78 ± 0.12
	rCBV vs. [DA]	−0.22	−0.21 ± 0.22
	rCBV vs. [CA]	−0.50	−0.34 ± 0.26
M1	[DA] vs. [CA]	0.06	0.00 ± 0.14
	rCBV vs. [DA]	0.17	−0.04 ± 0.10
	rCBV vs. [CA]	0.17	0.24 ± 0.18
	ipsi- vs. contra-rCBV	0.98	0.72 ± 0.12

Product–moment cross-correlation coefficient ρ among cocaine, dopamine, and rCBV time courses for each cohort.

[15,30]-min window ($r = -0.68$, $P = 0.06$, NS) and between rCBV and [CA] in the [0,15]-min window ($r = -0.66$, $P = 0.07$, NS) were observed.

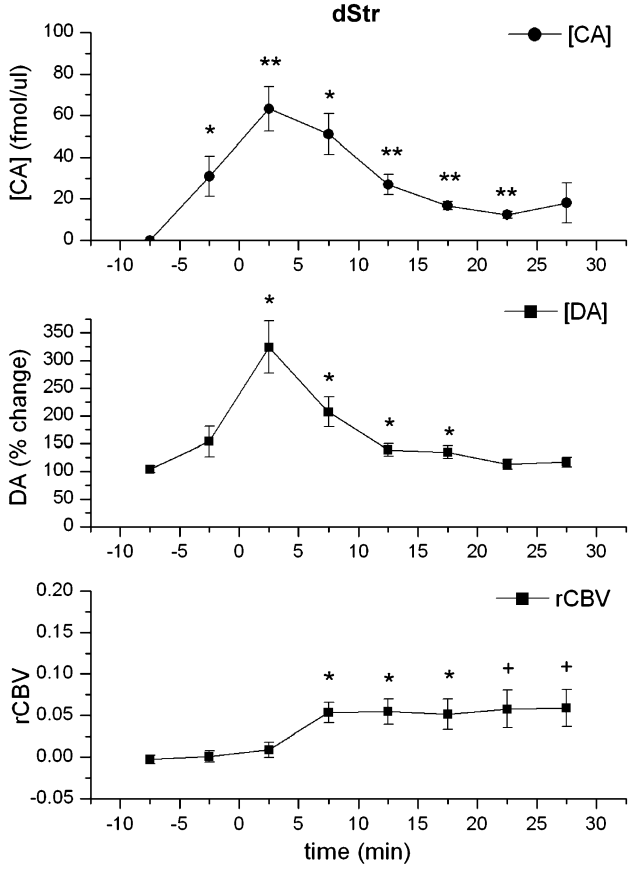

Fig. 3. Neurochemical and rCBV changes from the dorsal striatum cohort ($n = 8$). (a) Cocaine. (b) Percentage changes in dopamine relative to baseline. Mean basal [DA] was 0.50 ± 0.05 fmol/μl. (c) Local (striatum) and reference (M1) rCBV values, rebinned to 5-min intervals for direct comparison. All graphs show mean ± SEM across animals, with the data shown at the center of their corresponding time window. Time points where values are significantly different than baseline are indicated ($^{+}P < 0.05$, $^{*}P < P_{FDR}$, $^{**}P < 0.001$).

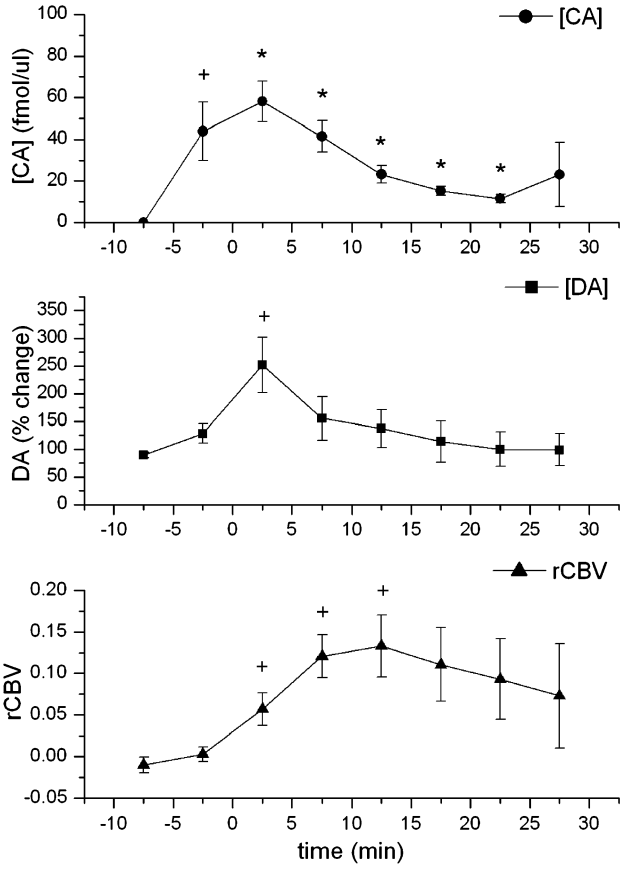

Fig. 4. Neurochemical and rCBV changes from the mPFC cohort ($n = 5$). (a) Cocaine. (b) Percentage changes in dopamine relative to baseline. Mean basal [DA] was 0.29 ± 0.13 fmol/μl. (c) Local (mPFC) and reference (M1) rCBV values, rebinned to 5-min intervals for direct comparison. All graphs show mean ± SEM across animals, with the data shown at the center of their corresponding time window. Time points where values are significantly different than baseline are indicated ($^{+}P < 0.05$, $^{*}P < P_{FDR}$, $^{**}P < 0.001$).

Cocaine did not induce dopamine changes in the motor cortex, despite strong rCBV response

In the motor cortex group, despite a local cocaine profile similar to that measured in the striatum and mPFC, and a robust local rCBV response, no significant change in local [DA] was observed at any time point (Fig. 5). This observation was systematic; all individual animals showed a flat [DA] response in the presence of strong cocaine changes in the range 52–115 fmol/μl (peak [CA]). The cross-correlation coefficients were correspondingly low (Table 1). Moreover, no significant correlations were found between the amplitude of the rCBV response and the [CA] changes.

Dopamine baseline values were stable in all cohorts; ANOVA analysis revealed no significant effect of time [striatum: $F(7,8) = 0.31$, $P = 0.95$, NS; mPFC: $F(4,8) = 1.08$, $P = 0.40$, NS; M1: $F(7,8) = 0.75$, $P = 0.64$, NS].

Discussion

The present study was designed to test the hypothesis of a tight coupling between dopamine and rCBV changes (Chen et al.,

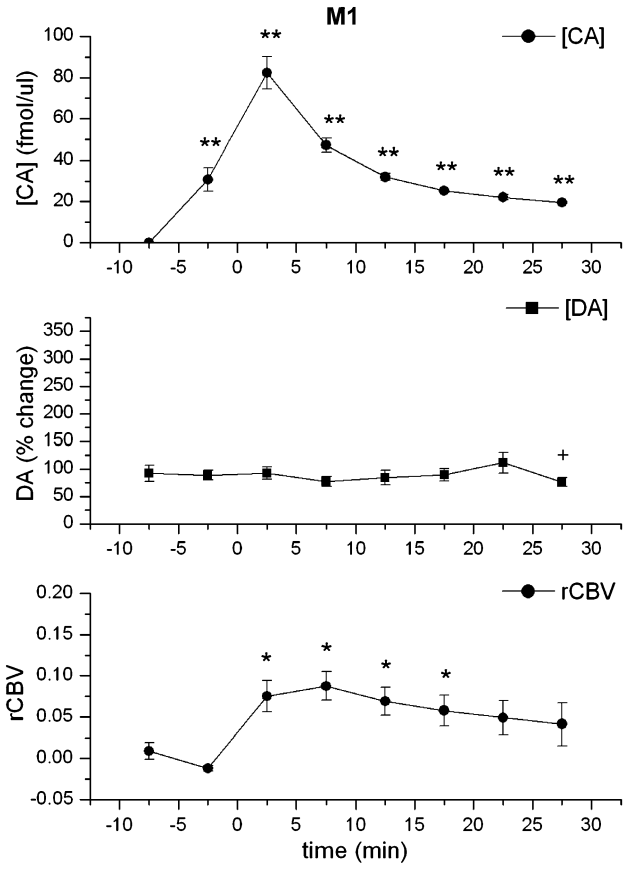

Fig. 5. Neurochemical and rCBV changes from the motor cortex cohort (n = 8). (a) Cocaine. (b) Percentage changes in dopamine relative to baseline. Mean basal [DA] was 2.24 ± 0.07 fmol/μl. (c) Local (M1) and reference (dorsal striatum) rCBV values, rebinned to 5-min intervals for direct comparison. All graphs show mean ± SEM across animals, with the data shown at the center of their corresponding time window. Time points where values are significantly different than baseline are indicated ($^{+}P < 0.05$, $^{*}P < P_{FDR}$, $^{**}P < 0.001$).

1997, 1999) following acute intravenous cocaine challenge under anesthetic conditions known to produce a robust phMRI response (Marota et al., 2000). In particular, we investigated the relationship between the different rCBV time courses observed in the striatum, mPFC, and motor cortex and the temporal profiles of local dopamine changes. The rCBV time courses we observed were consistent with those presented in the literature using the same technique (Marota et al., 2000) and with rCBF changes measured by autoradiography (Stein and Fuller, 1993). Acute intravenous cocaine administration in man also resulted in similar blood oxygenation level-dependent (BOLD) MRI response profiles within a 10-min postchallenge time window (Breiter et al., 1997).

Increasing the time resolution of the microdialysis acquisition to 5-min sampling intervals allowed the dynamics of [CA] and [DA] to be elucidated on the time scale of the fast pharmacokinetics of intravenous cocaine. However, to perform the experiments with the animal in the center of the MR magnet bore, a 60-cm long outflow tube was required. The combination of this and the flow rate was such that three consecutive samples (15 min) were present in the tube at any one time. Some limited diffusion between adjacent sample windows in the presence of cocaine might explain increased dopamine levels in the sample immediately before cocaine injection. Nevertheless, the rapid concentration increases and decreases in subsequent samples showed that fast changes in monoamine concentration could be captured reliably. Indeed, the presence of significant cocaine and increased dopamine in the [−5,0]-min window implies that strong changes occur at the beginning of the [0,5]-min period.

The main findings here demonstrated that the time courses followed by rCBV are not tightly coupled to [DA] changes in any of the three regions investigated. While dopamine changes in the mPFC and striatum closely tracked the local pharmacokinetics of cocaine, peaking within the first 5-min following injection, the rCBV response was slower than dopamine changes. Despite the mismatch in temporal profile, we observed a negative correlation between the amplitudes of dopamine and rCBV changes in the mPFC and a positive trend in the striatum. Thus, greater dopamine increases appear to be associated with a weaker hemodynamic response in the mPFC; this is consistent with the inhibitory action of both dopaminergic pathways and locally administered dopamine on both spontaneous and evoked activity in the mPFC (Ferron et al., 1984; Gulledge and Jaffe, 1998; Mantz et al., 1988; Pirot et al., 1992). However, the mismatch in temporal profile suggests that the association between any action of dopamine—excitatory or inhibitory—and the phMRI response is complex, and that direct vasoactivity of dopamine is unlikely to be the dominant mechanism underlying rCBV increases.

The finding of no [DA] changes in the motor cortex was unexpected given the strong cortical rCBV response to cocaine, as well as to more specific dopaminergic stimuli such as d-amphetamine that produce a similar rCBV activation pattern (Jenkins et al., 2003). This demonstrates that the rCBV response in the motor cortex is not driven by local dopamine changes, an important finding for the interpretation of phMRI studies targeting the dopaminergic system, given that cortical regions are associated with the strongest hemodynamic response. The response in the motor cortex could reflect local changes in other neurotransmitters involved in the response to cocaine, not measured in the present study. Although often used as a probe of the dopamine system, cocaine elicits a complex CNS response and also inhibits reuptake of serotonin (5-HT) and norepinephrine (NE) (Andrews and Lucki, 2001; Reith et al., 1997; Ritz et al., 1987). Indeed, the dopamine D_1 antagonist SCH-23390 and the broad-spectrum 5-HT antagonists methiothepin and metergoline have all been shown to block the rCBV response to cocaine (Mandeville et al., 2002). Alternatively, the hemodynamic response could be driven by local metabolic demand. Autoradiography studies have shown increases in cerebral $rCMR_{glc}$ following both intravenous and intraperitoneal cocaine challenge in the unanesthetized rat. In an intravenous study (Porrino et al., 1988), significant increases in local glucose utilization were restricted to the mPFC and nucleus accumbens at 0.5 mg/kg iv but spread to extrapyramidal and neocortical regions at higher doses. In another study, using a dose of 30 mg/kg ip (Thomas et al., 1996), metabolic activity was significantly increased in regions including both the motor cortex and dorsal striatum, and coronal images through the substantia nigra (z_{bregma} −5.8 mm) showed strong increases in cortical areas and in a number of mesencephalic nuclei that also show strong rCBV responses. This evidence, together with our results, corroborates the idea that the cortical rCBV changes induced by cocaine challenge reflect neuronal activity, perhaps driven by afferent projections from

dopaminergic subcortical structures, rather than the local vasoactivity of dopamine.

We often observed a rapid rCBV decrease upon cocaine injection, as can be seen in the striatum group time course (Fig. 1C). It might be speculated that this be due to an initial surge of dopamine release exerting a direct vasoconstrictive action. However, this rCBV decrease was also observed in individual animals in the motor cortex in the absence of local dopamine changes, suggesting that this negative dip is more likely due to the transitory MABP increase upon cocaine injection.

We also found a higher basal [DA] in the motor cortex than in the more commonly investigated striatum and mPFC. Possible regional differences in the dopamine transporter (DAT) density (Richtand et al., 1995) might partially explain these data; differences between the dorsal striatum, prelimbic, and anterior cingulate cortices have been demonstrated (Sesack et al., 1998). Moreover, there is some evidence that dopamine levels in the striatum can be altered by halothane anesthesia (Adachi et al., 2000, 2001), and this could also be true of other brain regions. Nevertheless, the conclusion that the strong rCBV changes observed in the motor cortex under these experimental conditions do not reflect local [DA] changes remains valid.

In summary, we have shown that neither the pharmacokinetic profile of cocaine nor the time course of dopamine accurately models the rCBV response to acute intravenous cocaine. While the dopaminergic system is clearly implicated in the neurobiological response to cocaine, hemodynamic changes visible by phMRI do not appear to be solely driven by local extracellular dopamine levels. This suggests that the phMRI signals observed following cocaine challenge do not merely represent direct vasoactivity of dopamine and corroborates the hypothesis that they reflect changes in neural activity. The combination of phMRI and in situ microdialysis will be of great value in elucidating the relationship between the phMRI response to psychoactive drugs and underlying neurochemical changes.

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